A2 cont

(i) introducing said nucleic acids into [an] attenuated bacteria, said bacteria having an attenuating factor which will result in lysis of the bacteria after entry into said cell; and

(ii) administering said bacteria to said cell.

REMARKS

Claims 28-33 and 44 are under examination. Reconsideration is requested. Claims 28 and 44 have been amended to more particularly recite preferred embodiments of the invention. Support for the amendments can be found throughout the specification. No new matter has been added.

The Invention

A diagram of the present invention is attached for clarification and convenience in understanding Applicants' comments.

Step 1 in the diagram is the construction of the attenuated strain. The present specification describes as an example the construction of a Δ*asd* Shigella strain 2457T, 15D, and the placement by electroporation of a reporter mammalian expression plasmid into the bacterium. The *asd* gene is an essential gene required by the bacterium to synthesize its cell wall. This attenuating factor is used in the present invention as a method to lyse the bacterium for release of the plasmid DNA into the cytoplasm, in contrast to its previous use as a means to

stabilize the carriage of plasmids. Mutations in other genes required for cell wall synthesis can be similarly used, as will be apparent to persons of skill in the art.

Step 2 in the diagram is adherence and invasion of the bacteria. As part of their interaction with a host, Shigella adhere to and invade epithelial cells of the gut. This process also occurs *in vitro* with a wide variety of cell lines.

Step 3 is release from the endocytic vacuole. After adherence to and invasion of the mammalian cell, Shigella breaks the bilayer of the endocytic vacuole down, so that the bacteria are now within the cell cytoplasm.

Step 4 is division and spreading. After release from the endocytic vacuole, Shigella will begin the process of dividing and spreading to adjacent cells. However, strain 15D contains a mutation in its asd gene. Therefore, it cannot synthesize all of the needed components of its cell wall and lysis of the cell results.

Step 5 is death of the bacterium and release of the plasmid DNA. Upon lysis of the bacterium, the carried expression plasmid is delivered to the cell cytoplasm.

Step 6 is transcription and translation. The expression plasmid that is now free in the cell cytoplasm travels to the nucleus and transcription and translation by the cell machinery follows.

Rejections Under 35 USC § 102/103

Claim 44 was rejected under 35 USC § 102(b) as being anticipated by Sansonetti et al. Claim 44 has been amended to more particularly recite the claimed invention. To the extent to which the rejection over Sansonetti might be considered to apply to the amended claim, it is traversed for the following reasons.

The technology of the present invention involves a process in which bacteria that have been specifically mutated to undergo lysis can be utilized to deliver intact plasmid DNA to mammalian cells. Previously used technologies have utilized attentuated bacteria to express proteins/peptides which are capable of inducing an immune response in the host *via* carried plasmids or on the chromosomes of the bacteria. In the present invention, the bacterium does not express the foreign protein/peptide, rather the mammalian cell does. While Sansonetti discloses a method for delivery of functional nucleic acids into bacteria and administering bacteria to a cell, Sansonetti does not disclose a method for using the bacteria to deliver the DNA to be expressed by the target cell. The nucleic acid is added to the bacteria to change the phenotype (characteristics) of the bacteria. Thus, in Sansonetti, the nucleic acid (DNA) is being expressed within the bacterium, and not by the mammalian cell. Accordingly, Sansonetti does not anticipate the present invention. Withdrawal of the rejection is respectfully requested.

Claim 44 was rejected under 35 USC § 102(e) as being anticipated by Brey et al. To the extent that this rejection may be considered applicable to the amended claim, it is traversed for the following reasons.

The Examiner has indicated that Brey et al. disclose a method for the delivery of functional nucleic acids into a cell using bacteria comprising introducing said nucleic acids into an attenuated (non-virulent) bacteria and administering said bacteria to a cell. Applicants submit that the teaching of Brey et al., like the teaching of Sansonetti et al., does not disclose the administration of bacteria which lyse after entering the cell and thereby will deliver DNA in a form which will be expressed by the mammalian cell. Thus, Brey et al. does not anticipate the presently claimed invention. Reconsideration and withdrawal of the rejection are respectfully requested.

Claims 28, 29, and 31-33 were rejected under 35 USC § 103(a) as being unpatentable over Brey et al. The Examiner has indicated that Brey et al. disclose a method for delivering DNA to a cell, said method comprising introducing said DNA into attenuated Salmonella. It is the Examiner's position that it would have been obvious to a person of skill in the art to substitute Shigella strains in view of the teachings of Brey et al. Applicants note that, as in the previous § 102 rejections, the teachings of Brey et al. lack the essential feature that the administered bacteria have been modified so as to lyse when they

enter the target cell, thereby delivering the DNA in a form which will be functionally incorporated into the target cell. Accordingly, it is submitted that the claims are not obvious in view of Brey et al. Withdrawal of the rejection is respectfully requested.

Claim 30 was rejected under 35 USC § 103(a) as being unpatentable over Brey et al. in view of Curtiss et al. It is the Examiner's position that it would have been obvious to a person of skill in the art to substitute Shigella strains for the Salmonella strains used by Brey et al., and further to have used the ask-strain of Shigella as disclosed by Curtiss et al., since such mutations were known in the art to cause attenuation.

Claim 30 was rejected under 35 USC § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains to make and/or use the invention. It is the Examiner's position that it is unclear if the disclosure is sufficiently repeatable to avoid the need of a deposit, and that it is unclear if the disclosure alone is sufficient to provide an enabling diclosure because it is unknown if the starting materials were readily available to the public at the time of the invention. Applicants submit in this regard that the microorganisms of claim 30 has been deposited at the ATCC under assession no. 55710, and that the required assurances as to the

continued availability of the microorganisms will be provided in due course.

Claim 44 was rejected under 35 USC § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. It is the Examiner's position that the phrase "functional nucleic acids" is unclear. This rejection is traversed for the following reasons.

As indicated, *inter alia*, at page 1, lines 18-19, a functional nucleic acid is one which is capable of directing the cell (e.g. the target mammalian cell of the invention) to produce antigens or other functional molecules. The term functional is thus intended to mean that the nucleic acid can perform the normal functions expected of nucleic acids which result in the production of expected end products. It is respectfully submitted that this is not indefinite, and will be clear to persons of skill in the art upon reading the claims and specificaion. Reconsideration and withdrawal of the rejection are requested.

All rejections having been addressed, it is believed that this application is in condition for allowance, and Notice to that effect is respectfully requested.

Respectfully submitted,

CUSHMAN DARBY & CUSHMAN
INTELLECTUAL PROPERTY GROUP OF
PILLSBURY MADISON & SUTRO, L.L.P.

By: _____

Ann S. Hobbs, Ph.D.
Reg. No. 36,830
Tel: (202) 861-3063

1100 New York Avenue
Ninth Floor
Washington, D.C. 20005